HHS Public Access

Author manuscript

Nutr Cancer. Author manuscript; available in PMC 2016 January 28.

Published in final edited form as:

Nutr Cancer. 2015 ; 67(2): 292–304. doi:10.1080/01635581.2015.990568.

MAPK genes interact with diet and lifestyle factors to alter risk of breast cancer: The Breast Cancer Health Disparities Study

Martha L. Slattery[1], **Abbie Lundgreen**[1], **Esther M. John**[2], **Gabriela Torres-Mejia**[3], **Lisa Hines**[4], **Anna R. Giuliano**[5], **Kathy B. Baumgartner**[6], **Mariana C. Stern**[7], and **Roger K. Wolff**[1]

[1]University of Utah, Department of Medicine, 383 Colorow, Salt Lake City, Utah 84108

[2]Cancer Prevention Institute of California, Fremont, CA, USA, and Division of Epidemiology, Department of Health Research and Policy and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA

[3]Instituto Nacional de Salud Pública, Centro de Investigación en Salud Poblacional, Av. Universidad No. 655, Col. Sta. Ma. Ahuacatitlán, Cuernavaca Morelos CP 62100, México

[4]University of Colorado at Colorado Springs, Department of Biology, 1420 Austin Bluffs Parkway, Colorado Springs, CO 80918

[5]Moffitt Cancer Center and Research Institute, Tampa, Florida 33612

[6]Department of Epidemiology and Population Health, School of Public Health & Information Sciences, University of Louisville, Louisville, KY

[7]Department of Preventive Medicine, Keck School of Medicine of USC, University of Southern California, Los Angeles, California 90089

Abstract

Mitogen-activated protein kinases (MAPK) are integration points for multiple biochemical signals. We evaluated 13 *MAPK* genes with breast cancer risk and determined if diet and lifestyle factors mediated risk. Data from three population-based case-control studies conducted in Southwestern United States, California, and Mexico included 4183 controls and 3592 cases. Percent Indigenous American (IA) ancestry was determined from 104 Ancestry Informative Markers. The adaptive rank truncated product (ARTP) was used to determine the significance of each gene and the pathway with breast cancer risk, by menopausal status, genetic ancestry level, and ER/PR strata.

MAP3K9 was associated with breast cancer overall (P_{ARTP}=0.02) with strongest association among women with the highest IA ancestry (P_{ARTP}=0.04). Several SNPs in *MAP3K9* were associated with ER+/PR+ tumors and interacted with dietary oxidative balance score (DOBS), dietary folate, body mass index (BMI), alcohol consumption, cigarette smoking, and a history of diabetes. *DUSP4* and *MAPK8* interacted with calories to alter breast cancer risk; *MAPK1* interacted with DOBS, dietary fiber, folate and BMI; *MAP3K2* interacted with dietary fat; and *MAPK14* interacted with dietary folate and BMI. The patterns of association across diet and lifestyle factors with similar biological properties for the same SNPs within genes provide support for associations.

Address correspondence to Dr. Slattery University of Utah, Department of Medicine, 383 Colorow, Salt Lake City, Utah 84108.

Keywords

Breast Cancer; Indigenous Ancestry; MAPK; *MAP3K9*; diet; diabetes; body size; polymorphisms

Mitogen-activated protein kinases (MAPK) act as integration points for multiple biochemical signals and are involved in a variety of cellular processes, including cell proliferation, differentiation, transcription regulation and development [1]. By phosphorylating transcription factors, kinases and other enzymes, they influence gene expression, metabolism, cell division, morphology, and survival. Each MAPK pathway is a three-tiered cascade that includes a MAP kinase kinase kinase (MAP3K, MEKK, or MKKK), Map kinase kinase (MAP2K, MEK, or MKK), and the MAP kinase (MAPK). MAPKs are attenuated by dual specificity MAPK phosphatases (MKPs or DUSP). Three of the major MAPK pathways are extracellular regulated kinases (ERK), c-Jun-N-terminal kinases (JNKs) sometimes called stress-activated protein kinases (SAPK), and p38 [2]. Deregulation of the MAPK pathways has been associated with a variety of diseases such as cancer and type-2 diabetes and with inflammation [3–5].

MAPK pathways are activated by various environmental stimuli, cytokines, and hormones. ERK1 and ERK2 are activated by stimuli such as growth factors and cytokines [1]. The JNK pathway is involved in regulating responses to stress, inflammation, and apoptosis and are activated by radiation, environmental stresses, and growth factors. The JNK pathway has been shown to be involved in the development of obesity and type 2 diabetes [3,4]. The *p38* MAPKs have been linked to autoimmunity in humans and are activated by chemical stresses, hormones, cytokines including IL-1 and TNF, and oxidative stress [1,2]. MAPK mediate several signaling pathways associated with cancer, including IL1, IκBK, NFκB, PPARγ, TNFα, and TGFβ, and BMP [6–10].

Dietary factors likely affect many of these pathways through their antioxidant and pro-oxidant properties as well as possibly influencing growth factors and insulin through energy-contributing nutrients [11]. Lifestyle factors, including body size, cigarette smoking, alcohol, and diabetes may also affect the MAPK signaling pathway through their association with inflammation, oxidative stress, and insulin. Body size has been associated with breast cancer with most studies showing an inverse association with pre-menopausal women and a slight increased risk among post-menopausal women [12–15]; in Latina women obesity has been shown to be inversely associated with both pre- and post-menopausal [16,17]. Cigarette smoking has been inconsistently associated with breast cancer risk [18,19], while alcohol has been shown to slightly increase risk in most populations [20–23]. Few studies have evaluated diabetes robustly with breast cancer risk, although it has been hypothesized that insulin resistance influences breast cancer risk [24–27]. Associations with dietary intake varies and studies have suggested differences in effect for several nutrients among Latina women [20,28].

In this study we evaluated the association between genetic variation in key *MAPK* genes and the risk of breast cancer in a genetically admixed population living in the Southwestern United States, California, and Mexico. We investigated associations between the *MAPK* genes and the risk of breast cancer was modified by potential activators of the pathway such

as dietary factors, body mass index (BMI), alcohol intake, cigarette smoking status, and having been diagnosed with diabetes. We hypothesize that *MAPK* genes are associated with breast cancer and that these associations are modified by diet and lifestyle factors as well as by IA ancestry and ER/PR tumor status.

Methods

The Breast Cancer Health Disparities Study includes participants from three population-based case-control studies, the 4-Corners Breast Cancer Study (4-CBCS), the Mexico Breast Cancer Study (MBCS), and the San Francisco Bay Area Breast Cancer Study (SFBCS) who completed an in-person interview and who had a blood or mouthwash sample available for DNA extraction [17,29–31]. All participants signed informed written consent prior to participation and each study was approved by the Institutional Review Board for Human Subjects.

4 Corner's Breast Cancer Study

Participants were NHW, Hispanic, or Native American women living in non-reservation areas in the states of Arizona, Colorado, New Mexico, or Utah at the time of diagnosis or selection [17]. Eligible female breast cancer cases were between 25 and 79 years of age with a histological confirmed diagnosis of *in situ* (n=337) or invasive cancer (n=1466) (ICDO sites C50.0-C50.6 and C50.8-C50.9) between October 1999 and May 2004. Controls were selected from the target populations and were frequency matched to cases on the expected ethnicity and 5-year age distribution. In Arizona and Colorado controls under age 65 years were randomly selected from a commercial mailing list; in New Mexico and Utah they were randomly selected from driver's license lists. In all states, women 65 years and older were randomly selected from Center for Medicare Services lists. Women were screened by telephone for eligibility and self-identified their race/ethnicity prior to study enrollment. Of cases contacted, 852 Hispanic, 22 American Indian, and 1683 NHW women participated. Of controls contacted, 913 Hispanic, 23 American Indian, and 1669 NHW women participated. Blood was collected and DNA extracted for 76% of participants in Arizona, 71% of participants in Colorado, 75% of participants in New Mexico, and 94% of participants in Utah.

Mexico Breast Cancer Study

Participants were between 28 and 74 years of age, living in one of three states, Monterrey, Veracruz and Mexico City, for the past five years as previously described [32]. Eligible cases were women diagnosed with either a new histologically confirmed *in situ* or invasive breast cancer between January 2004 and December 2007 at 12 participating hospitals from three main health care systems in Mexico, IMSS, ISSTE, and SS. In situ and invasive cancers were not distinguished in the study database. Controls were randomly selected from the catchment area of the 12 participating hospitals using a probabilistic multi-stage design. A total of 1000 cases and 1074 controls were recruited, and blood was collected and DNA extracted from 85% and 96% of women respectively.

San Francisco Bay Area Breast Cancer Study

Participants were Hispanic, African American, and NHW women aged 35 to 79 from the San Francisco Bay Area diagnosed with a first primary histologically confirmed invasive breast cancer between 1995 and 2002; controls were identified by random-digit dialing (RDD) [30,31]. This analysis was limited to women who participated in the biospecimen component of the parent study that was initiated in 1999 [33]. Eligible cases were Hispanic women diagnosed between April 1997 and April 2002 and a 10% random sample of NHW women diagnosed between April 1997 and April 1999. RDD controls were frequency-matched to cases based on race/ethnicity and the expected 5-year age distribution of cases. Women participated in a telephone screening interview that assessed study eligibility and self-identified race/ethnicity. DNA was available for 93% of cases and 92% of controls interviewed, including 1105 cases (793 Hispanics, 312 NHW) and 1318 controls (998 Hispanics, 320 NHW).

Data Harmonization

Data were harmonized across all study centers and questionnaires as previously described [29]. Women were classified as either pre-menopausal or post-menopausal based on responses to questions on menstrual history. Women who reported still having periods during the referent year (defined as the calendar year before diagnosis for cases or before selection into the study for controls) were classified as pre-menopausal. Women were classified as post-menopausal if they reported either a natural menopause or if they reported taking hormone therapy (HT) and were still having periods and were at or above the 95th percentile of age for those who reported having a natural menopause (i.e., >12 months since their last period).

Lifestyle variables included body mass index (BMI) calculated as self-reported weight (kg) during the referent year divided by measured height squared (m^2). Parity was defined as the number of total pregnancies. Cigarette smoking was evaluated as ever versus never having smoked cigarettes on a regular basis or more than 100 cigarettes. Those classified as having a history of diabetes reported being told by a doctor or health professional that they had diabetes or high blood sugar. A dietary oxidative balance score (DOBS) that included nutrients with anti- or pro-oxidative balance properties was developed as previously reported [34]. Dietary information was collected via a computerized validated diet history questionnaire for the 4-CBCS [35,36], a 104-item semi-quantitative Food Frequency Questionnaire (FFQ) in Mexico City [37], and the Block Food Frequency Questionnaire in SFBCS [38]. The food frequency questionnaire used in the 4-CBCS queries consumption of foods in major categories and if that is yes, then more detail about specific foods are obtained. For instance, a question would ask “Do you eat eggs?” If the response is yes, then details of types of eggs and related frequency and amount for each type were obtained. The FFQ asked a list of food items and participants provide information for each food item in the list. Given differences in food questionnaires, categories of consumption were study specific.

Genetic Data

DNA was derived from either whole blood or mouthwash samples obtained from study participants. A total of 7286 blood-derived and 637 mouthwash-derived samples were studied. Whole Genome Amplification (WGA) was applied to the mouthwash-derived DNA samples prior to genotyping. A tagSNP approach was used to characterize variation across candidate genes. TagSNPs were selected using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map and an r^2=0.8; minor allele frequency (MAF) >0.1; range= −1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin. For genes where a functional SNP was identified, that SNP was included in the platform. Additionally, 104 Ancestral Informative Markers (AIMs) were used to distinguish European and Native American ancestry in the study population [29]. All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.93% was attained (99.65% for WGA samples). We included 132 internal replicates that were blinded representing 1.6% of the sample set. The duplicate concordance rate was 99.996% as determined by 193,297 matching genotypes among sample pairs. In the current analyses we evaluated *DUSP4* (6 SNPs), *DUSP6* (1 SNP), *MAP2K1* (6 SNPs), *MAP3K1* (7 SNPs), *MAP3K2* (3 SNPs), *MAP3K3* (2 SNPs), *MAP3K7* (6 SNPs), *MAP3K9* (19 SNPs), *MAPK1* (6 SNPs), *MAPK3* (1 SNP), *MAPK8* (4 SNPs), *MAPK12* (2 SNPs), and *MAPK14* (9 SNPs). Genes and SNPs are described in online Supplements 1 and 2.

Tumor Characteristics

Data for ER/PR tumor status were available from local tumor registries for cases from the 4-CBCS and the SFBCS for 1019 (69%) non-Hispanic white (NHW) and 977 (75%) Hispanic/Native American (NA) women.

Statistical Methods

The program STRUCTURE was used to compute individual ancestry for each study participant assuming two founding populations [39,40]. A two-founding population model was used. Assessment across categories of ancestry was done using cut-points based on the distribution of genetic ancestry in the control population. Three strata, ≤28%, >28–70%, and >70%, were used to evaluate associations by level of Indigenous American (IA) ancestry. Cut-points were chosen to maximize power within the three ancestry groups while maintaining the ability to discriminate unique ancestry groups.

P values are based on chi-square tests when comparing number of cases to controls by categorical variables and on Wilcoxon Rank Sum tests when measuring differences in median values. Genes and SNPs were assessed for their association with breast cancer risk by strata of menopausal status and genetic ancestry in the whole population and by ER/PR status for the SFBCS and 4-CBCS. Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC) unless otherwise noted. Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with SNPs, adjusting for five-year age categories (continuous), study center, genetic ancestry (continuous), BMI during referent year (continuous), and parity (continuous). Age and study center were matching variables and therefore adjusted in the

analysis. BMI and parity were included as adjustment variables given their association with breast cancer and possible association with genes being examined.The generalized logit link function was used when estimating risk by ER/PR status. Associations with SNPs were assessed assuming a co-dominant model. Based on the initial assessment, SNPs which appeared to have a dominant or recessive mode of inheritance were evaluated with those inheritance models in subsequent analyses.

We used the adaptive rank truncated product (ARTP) method that is based on a highly efficient permutation algorithm to determine the significance of association of each gene and of the pathway with breast cancer overall, by menopausal status, by genetic ancestry level, and by ER/PR strata. The gene *p* values were generated using the ARTP package in R, permuting outcome status 10,000 times while adjusting for age, BMI during referent year, and genetic ancestry [41,42]. We report both pathway and gene *p* values (P_{ARTP}) as an indicator of the importance of the gene and the overall pathway with breast cancer risk.

We examined if the association between SNPs and risk of breast cancer was different by menopausal status, ER and PR tumor status, level of IA ancestry, and diet and lifestyle factors. Diet and lifestyle factors were selected based on their potential to modify factors associated with oxidative stress, inflammation, growth factors, and/or insulin and categorized to test for interactions. For stratified analyses, tests for interactions were calculated using a Wald 1-degree of freedom (1-df) chi-square tests; overall SNP associations with breast cancer by ER/PR status are estimated using p values from 4-df Wald tests. Adjustments for multiple comparisons for stratified analyses within the gene used the step-down Bonferroni correction (i.e., Holm method) taking into account the correlated nature of the data using the SNP spectral decomposition method proposed by Nyholt and modified by Li and Ji [43,44]. We report both the unadjusted and adjusted *p* values for interactions between genes and diet and lifestyle factors.

Results

The majority of women were post-menopausal at the time of diagnosis (Table 1). Almost all women (over 99%) who self-identified as being NHW had low levels of IA ancestry (≤28%), while those who self-identified as being Hispanic or Native American or who lived in Mexico, had a range of IA levels, although the majority had intermediate and high IA ancestry (>28%). Almost 50% of NHW women reported never drinking alcohol, compared to 78% of Hispanic/IA controls and 73% of Hispanic/NA cases reported never drinking alcohol. Among Hispanic/NA women, total calories was significantly higher among cases than controls and dietary fiber, folate, vitamin E, and beta carotene were significantly lower among cases than controls.

The overall pathway was not statistically significant overall or for any admixture group. Only *MAP3K9* was significantly associated with breast cancer risk overall (P_{ARTP}=0.02) and for women with the highest level of IA ancestry (P_{ARTP}=0.04) (Table 2). Several *MAP3K9* SNPs were significantly associated with breast cancer among all women and/or by strata of IA ancestry (rs11628333, rs10483834, rs11622989, rs12883244, rs11158881, rs4902855, rs10143031, and rs11624934). *MAP3K3* rs3785574 and MAPK8 rs10508901

associations with breast cancer were significantly different across ancestry group although neither of the genes was statistically significant by the ARTP *p* value. We did not observe differences in breast cancer associations by menopausal status.

Significant differences in breast cancer risk were identified by ER/PR tumor status (Table 3).). The pathway P_{ARTP} was of borderline significance for ER−/PR− tumors (P_{ARTP}=0.06). *MAP3K3* was significantly associated with ER−/PR− tumors (P_{ARTP}=0.002) and *MAP3K3* rs3785574 was significantly associated with these tumors (OR 1.74, 95% CI 1.26, 2.39). *MAP3K9* was significantly associated with ER+/PR+ tumors (P_{ARTP}=0.01) based on significant associations with several SNPs (rs11622989, rs17176971, rs12883244, rs4902855, and rs11624934). *MAPK3* was significantly associated with ER+/PR− tumors (P_{ARTP}=0.048) with rs7698 being inversely associated with breast cancer risk (OR 0.65, 95% CI 0.43, 0.99).

Assessment of dietary factors that could modify associations between *MAPK* genes and breast cancer risk showed several significant interactions (Table 4). *DUSP4* (1 SNP), *MAP3K7* (1 SNP), *MAP3K9* (2 SNPs), *MAPK14* (1 SNP), and *MAPK1* (3 SNPs) interacted with the DOBS. High DOBS reduced breast cancer risk for those with the homozygote common genotype. *DUPS4* (3 SNPs), *MAP3K1* (1 SNP), *MAPK8* (2 SNPs), and *MAPK3* (1 SNP) interacted with total caloric intake; fewer calories generally reduced breast cancer risk among women with the homozygote rare alleles. *MAP3K2* (2 SNPs) interacted with dietary fat; a high fat diet and having the CC genotype of rs12613413 increased breast cancer risk, while a high fat diet decreased breast cancer risk among women with the TT genotype of rs6732279. *MAPK8* (1 SNP) and *MAPK1* (4 SNPs) interacted with dietary fiber; high intake of dietary fiber generally reducing breast cancer risk among women with the homozygote common genotype. Dietary folate interacted with *DUSP4* (1 SNP), *MAP3K7* (1 SNP), *MAP3K9* (4 SNPs), *MAPK8* (1 SNP), *MAPK14* (2 SNPs), and *MAPK1* (3 SNPs); reduced breast cancer risk was observed for the homozygote common genotype in the presence of high folate..

When evaluating the interaction of *MAPK* genes and BMI we saw different sets of genes interacting with BMI to alter risk of pre-menopausal breast cancer versus post-menopausal breast cancer (Table 5). Among pre-menopausal breast cancer cases *DUSP4* (1 SNP), *MAP3K9* (5 SNPs), *MAPK8* (1 SNP), and *MAPK1* (2 SNPs) interacted with BMI, while among post-menopausal women BMI interacted with *MAPK1* (1 SNP), *MAP3K3* (1 SNP), *MAP3K9* (1 SNP), and *MAPK14* (2 SNPs). *MAP3K9* rs11622989 and rs12883244 interacted with both alcohol intake and cigarette smoking. High alcohol intake was associated with increased risk among women with the homozygote common genotype of *DUSP4* rs474824; cigarette smoking most strongly increased risk among women with the homozygote rare genotype of *MAPK14* rs13196204. A history of diabetes interacted with 11 *MAP3K9* SNPs to alter breast cancer risk. A history of diabetes was associated with increased risk of breast cancer among those with the homozygote rare genotype for rs11844774, rs11622989, rs12883244, rs1115881, rs4902855, and rs17108548. For *MAP3K9* rs11625206, rs11628333, rs10143031, rs8022269, and rs11624934, a history of diabetes in conjunction with the homozygote common allele genotype were associated with increased breast cancer risk.

Discussion

Although the overall MAPK pathway was not statistically significant, several *MAPK* genes were associated with breast cancer risk. *MAP3K9* appeared to make the largest contribution to risk through its overall effect on breast cancer risk that was stronger with increasing level of IA. Several SNPs in *MAP3K9* were associated with ER+/PR+ tumors specifically and showed interaction with DOBS, folate, obesity, alcohol intake, cigarette smoking, and a history of diabetes. In addition to *MAP3K9*, several other *MAPK* genes had multiple SNPs that jointly altered breast cancer risk with diet and lifestyle exposures. The patterns of association across diet and lifestyle factors with similar biological properties were similar for the same SNPs within genes, providing support that associations may be more than chance findings.

There is a continuum of decreasing breast cancer incidence rates across the spectrum of European to IA ancestry [29], hence our focus on differences in breast cancer risk by genetic ancestry. The admixed population of women living in the Southwestern United States, California, and Mexico included in this study allows us to examine this continuum. We observed the strongest associations for *MAP3K9*, the only gene with overall statistical significance, among those with the highest IA ancestry. For most SNPs we observed a continuum of risk across ancestry groups. *MAP3K9* was most strongly associated among women with the highest IA ancestry and multiple SNPs in this gene interacted with a history of diabetes to alter risk of breast cancer. At a population level, rates of diabetes and metabolic syndrome are higher among Hispanic, Native American and Mexican women than among NHW women [45–47]. While assessment of interaction of diet and lifestyle factors within ancestry groups would be desirable, our power was limited to meaningfully evaluate these 3-way interactions.

Several patterns of association emerged when evaluating interactions between dietary variables and MAPK genes and breast cancer risk. For example significant interactions were observed with breast cancer risk for *MAPK1* and DOBS, dietary fiber and folate; *DUSP4* rs2056025 interacted with both DOBS and dietary folate; *MAPK8* rs10508901 interacted with total calories, fiber, and folate intake. Additionally, directions of association for high and low risk genotype and high and low risk lifestyle group were similar for factors expected to have similar mechanisms, such as DOBS, folate, and fiber having a similar effect, but opposite of those observed for total calories. Patterns of interaction by lifestyle factors also showed consistency across SNPs. For example four of the five SNPs in *MAP3K9* shown to interact with pre-menopausal obesity also interacted with a history of diabetes. The two *MAP3K9* SNPs interacting with alcohol intake also interacted with cigarette smoking.

These patterns of association support the reported mechanisms of MAPK genes that include activation by stimuli such as growth factors, inflammation, cytokines, and stress [1]. The JNK pathway, which was associated with breast cancer risk in these data, is involved in regulating responses to stress, inflammation, and apoptosis and is activated by radiation, environmental stresses, and growth factors. *MAP3K9* appeared to be one of the most important *MAPK* genes with breast cancer in our population, both in terms of independent

risk and its interactive effects with diet and lifestyle factors. *MAP3K9*, also known as mixed-lineage kinase 1 (MLK1), is instrumental in the regulation of the JNK pathway that is associated with normal and malignant cellular growth and division [48]. Other genes that regulate the JNK and ERK pathways, including *MAP3K7* and *MAP3K*, also showed frequent interaction with diet and lifestyle factors. It is possible that response to diet and lifestyle factors is influenced by variation in genes at the activation point of these pathways. Dietary factors that influence oxidative balance may modify the effects of genes that respond to oxidative stress and inflammation. Cigarette smoking also could influence oxidative stress and importantly influence the effects of these genes to respond to stress. It could be further hypothesized that having a homozygote variant genotype of *MAP3K9* makes individuals more sensitive to the effects of obesity or diabetes resulting in activation of JNK-signaling pathway that in turn regulates cell growth, differentiation, and apoptosis and ultimately cancer risk. The JNK pathway has been shown previously to be involved in the development of obesity and type 2 diabetes [3]; our data suggest significant interaction between BMI and a history of diabetes with *MAP3K9*. *MAP3K7* is associated with transforming-growth factor β and bone morphogenetic protein signaling, both of which have been shown to influence breast cancer risk [49–51]; *MAP3K1* and *MAP3K3* enhance transcription of NFκB which is a key regulator of inflammatory response and associated with numerous cancers.

Few studies have examined genetic variation in *MAPK* genes and risk of cancer in general or breast cancer specifically. The variant allele of *MAP3K1* rs889312 has been associated with increased breast cancer in a GWAS of European women [52] and among women with ER− tumors [53], although we did not observe a significant association with this SNP. Studies that evaluated interaction between this SNP and BMI did not observe a significant association with breast cancer risk [54]. However, SNPs in *MAPK* genes have been shown to interact with diet and lifestyle factors to alter colon cancer risk [55,56]. A study of breast tumors by Stephens and colleagues [57], concluded that *MAP3K1* may harbor an important driver mutation. Hori and colleagues showed that ERα is regulated by MAPK and breast tumors that overexpressed ERK1, JNK1, and p38 proteins had more invasive tumor growth [58]. Given the biological role of *MAPK* genes there is support for an association, although previous studies have not examined polymorphisms in these genes and breast cancer risk.

The study has several strengths, including a large sample of Hispanic, NHW, and Mexican women, the assessment of AIMs that allowed examination of the continuum of European to IA ancestry, and our ability to look at interactions of key diet and lifestyle factors with these genes. While the information provided is novel and insightful to the pathways being studied, other *MAPK* genes and other diet and lifestyle factors that we did not have data on also may contribute to breast cancer risk and further illuminate these findings. A strength is our utilization of ARTP to evaluate the overall pathway and gene associations. This statistical method weighs the importance of the gene. Unfortunately ARTP has not been modified at this time to evaluate gene*environment interactions. Additionally, although we have limited information on functionality of SNPs associated with breast cancer, identification of similar associations for multiple SNPs within genes and patterns of interaction across genes and diet and lifestyle factors provides support for observed associations. Differences in dietary

patterns by level of ancestry could influence ability to detect associations for various ancestry groups.

In conclusion, our findings suggest that several *MAPK* genes were associated with breast cancer risk, although *MAP3K9* appeared to make the largest contribution to breast cancer risk. Several *MAPK* genes and SNPs, especially in *MAP3K9*, interacted with DOBS, dietary folate and fiber, total calories, obesity, alcohol intake, cigarette smoking, and a history of diabetes. The patterns of association across diet and lifestyle factors with similar biological properties for the same SNPs within genes provide support for the associations. Our findings suggest that this pathway may be most important for those women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would also like to acknowledge the contributions of the following individuals to the study: Sandra Edwards for data harmonization oversight; Jennifer Herrick for data management and data harmonization; Erica Wolff and Michael Hoffman for laboratory support; Carolina Ortega for her assistance with data management for the Mexico Breast Cancer Study, Jocelyn Koo for data management for the San Francisco Bay Area Breast Cancer Study; Dr. Tim Byers for his contribution to the 4-Corners Breast Cancer Study; and Dr. Josh Galanter for assistance in selection of AIMs markers.

Funding: The Breast Cancer Health Disparities Study was funded by grant CA14002 from the National Cancer Institute to Dr. Slattery. The San Francisco Bay Area Breast Cancer Study was supported by grants CA63446 and CA77305 from the National Cancer Institute, grant DAMD17-96-1-6071 from the U.S. Department of Defense and grant 7PB-0068 from the California Breast Cancer Research Program. The collection of cancer incidence data used in this study was supported by the California Department of Public Health as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201000036C awarded to the Cancer Prevention Institute of California; and the Centers for Disease Control and Prevention's National Program of Cancer Registries, under agreement #1U58 DP000807-01 awarded to the Public Health Institute. The 4-Corners Breast Cancer Study was funded by grants CA078682, CA078762, CA078552, and CA078802 from the National Cancer Institute. The research also was supported by the Utah Cancer Registry, which is funded by contract N01-PC-67000 from the National Cancer Institute, with additional support from the State of Utah Department of Health, the New Mexico Tumor Registry, and the Arizona and Colorado cancer registries, funded by the Centers for Disease Control and Prevention National Program of Cancer Registries and additional state support. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute or endorsement by the State of California Department of Public Health, the National Cancer Institute, and the Centers for Disease Control and Prevention or their Contractors and Subcontractors. The Mexico Breast Cancer Study was funded by Consejo Nacional de Ciencia y Tecnología (CONACyT) (SALUD-2002-C01-7462).

References

1. Imajo M, Tsuchiya Y, Nishida E. Regulatory mechanisms and functions of MAP kinase signaling pathways. IUBMB life. 2006; 58:312–317. [PubMed: 16754324]
2. Qi M, Elion EA. MAP kinase pathways. J Cell Sci. 2005; 118:3569–3572. [PubMed: 16105880]
3. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, et al. A central role for JNK in obesity and insulin resistance. Nature. 2002; 420:333–336. [PubMed: 12447443]
4. Lee YH, Giraud J, Davis RJ, White MF. c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem. 2003; 278:2896–2902. [PubMed: 12417588]
5. Kamata H, Honda S, Maeda S, Chang L, Hirata H, et al. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell. 2005; 120:649–661. [PubMed: 15766528]

6. Lubos E, Kelly NJ, Oldebeken SR, Leopold JA, Zhang YY, et al. Glutathione peroxidase-1 deficiency augments proinflammatory cytokine-induced redox signaling and human endothelial cell activation. J Biol Chem. 2011; 286:35407–35417. [PubMed: 21852236]
7. Mulder KM. Role of Ras and Mapks in TGFbeta signaling. Cytokine Growth Factor Rev. 2000; 11:23–35. [PubMed: 10708950]
8. Nasim MT, Ogo T, Chowdhury HM, Zhao L, Chen CN, et al. BMPR-II deficiency elicits pro-proliferative and anti-apoptotic responses through the activation of TGFbeta-TAK1-MAPK pathways in PAH. Hum Mol Genet. 2012; 21:2548–2558. [PubMed: 22388934]
9. To SQ, Knower KC, Clyne CD. NFkappaB and MAPK signalling pathways mediate TNFalpha-induced Early Growth Response gene transcription leading to aromatase expression. Biochem Biophys Res Commun. 2013; 433:96–101. [PubMed: 23485457]
10. Burns KA, Vanden Heuvel JP. Modulation of PPAR activity via phosphorylation. Biochim Biophys Acta. 2007; 1771:952–960. [PubMed: 17560826]
11. Hu R, Kong AN. Activation of MAP kinases, apoptosis and nutrigenomics of gene expression elicited by dietary cancer-prevention compounds. Nutrition. 2004; 20:83–88. [PubMed: 14698020]
12. Cappellani A, Di Vita M, Zanghi A, Cavallaro A, Piccolo G, et al. Diet, obesity and breast cancer: an update. Front Biosci (Schol Ed). 2012; 4:90–108. [PubMed: 22202045]
13. Lahmann PH, Hoffmann K, Allen N, van Gils CH, Khaw KT, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer. 2004; 111:762–771. [PubMed: 15252848]
14. Morimoto LM, White E, Chen Z, Chlebowski RT, Hays J, et al. Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control. 2002; 13:741–751. [PubMed: 12420953]
15. John EM, Phipps AI, Sangaramoorthy M. Body size, modifying factors, and postmenopausal breast cancer risk in a multiethnic population: the San Francisco Bay Area Breast Cancer Study. Springerplus. 2013; 2:239. [PubMed: 23762816]
16. Abdel-Maksoud MF, Risendal BC, Slattery ML, Giuliano AR, Baumgartner KB, et al. Behavioral risk factors and their relationship to tumor characteristics in Hispanic and non-Hispanic white long-term breast cancer survivors. Breast Cancer Research and Treatment. 2012; 131:169–176. [PubMed: 21822637]
17. Slattery ML, Sweeney C, Edwards S, Herrick J, Baumgartner K, et al. Body size, weight change, fat distribution and breast cancer risk in Hispanic and non-Hispanic white women. Breast Cancer Res Treat. 2007; 102:85–101. [PubMed: 17080310]
18. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, et al. Active smoking and breast cancer risk: original cohort data and meta-analysis. J Natl Cancer Inst. 2013; 105:515–525. [PubMed: 23449445]
19. Bjerkaas E, Parajuli R, Weiderpass E, Engeland A, Maskarinec G, et al. Smoking duration before first childbirth: an emerging risk factor for breast cancer? Results from 302,865 Norwegian women. Cancer Causes Control. 2013; 24:1347–1356. [PubMed: 23633026]
20. Beasley JM, Coronado GD, Livaudais J, Angeles-Llerenas A, Ortega-Olvera C, et al. Alcohol and risk of breast cancer in Mexican women. Cancer Causes Control. 2010; 21:863–870. [PubMed: 20155314]
21. Bowlin SJ, Leske MC, Varma A, Nasca P, Weinstein A, et al. Breast cancer risk and alcohol consumption: results from a large case-control study. Int J Epidemiol. 1997; 26:915–923. [PubMed: 9363510]
22. Brooks PJ, Zakhari S. Moderate alcohol consumption and breast cancer in women: from epidemiology to mechanisms and interventions. Alcohol Clin Exp Res. 2013; 37:23–30. [PubMed: 23072454]
23. Flatt SW, Thomson CA, Gold EB, Natarajan L, Rock CL, et al. Low to moderate alcohol intake is not associated with increased mortality after breast cancer. Cancer Epidemiol Biomarkers Prev. 2010; 19:681–688. [PubMed: 20160253]
24. Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care. 2003; 26:1752–1758. [PubMed: 12766105]

25. Redaniel MT, Jeffreys M, May MT, Ben-Shlomo Y, Martin RM. Associations of type 2 diabetes and diabetes treatment with breast cancer risk and mortality: a population-based cohort study among British women. Cancer Causes Control. 2012; 23:1785–1795. [PubMed: 22971998]
26. Dalamaga M. Obesity, insulin resistance, adipocytokines and breast cancer: New biomarkers and attractive therapeutic targets. World J Exp Med. 2013; 3:34–42. [PubMed: 24520544]
27. Eliassen AH, Tworoger SS, Mantzoros CS, Pollak MN, Hankinson SE. Circulating insulin and c-peptide levels and risk of breast cancer among predominately premenopausal women. Cancer Epidemiol Biomarkers Prev. 2007; 16:161–164. [PubMed: 17220346]
28. Murtaugh MA, Herrick J, Sweeney C, Guiliano A, Baumgartner K, et al. Macronutrient composition influence on breast cancer risk in Hispanic and non-Hispanic white women: the 4-Corners Breast Cancer Study. Nutr Cancer. 2011; 63:185–195. [PubMed: 21271459]
29. Slattery ML, John EM, Torres-Mejia G, Lundgreen A, Herrick JS, et al. Genetic variation in genes involved in hormones, inflammation and energetic factors and breast cancer risk in an admixed population. Carcinogenesis. 2012; 33:1512–1521. [PubMed: 22562547]
30. John EM, Horn-Ross PL, Koo J. Lifetime physical activity and breast cancer risk in a multiethnic population: the San Francisco Bay area breast cancer study. Cancer Epidemiol Biomarkers Prev. 2003; 12:1143–1152. [PubMed: 14652273]
31. John EM, Phipps AI, Davis A, Koo J. Migration history, acculturation, and breast cancer risk in Hispanic women. Cancer Epidemiol Biomarkers Prev. 2005; 14:2905–2913. [PubMed: 16365008]
32. Seinost G, Renner W, Brodmann M, Winkler M, Koppel H, et al. C677T mutation in the methylene tetrahydrofolate reductase gene as a risk factor for venous thrombotic disease in Austrian patients. Thrombosis research. 2000; 100:405–407. [PubMed: 11150582]
33. Pradhan SJ, Mishra R, Sharma P, Kundu GC. Quercetin and sulforaphane in combination suppress the progression of melanoma through the down-regulation of matrix metalloproteinase-9. Experimental and therapeutic medicine. 2010; 1:915–920. [PubMed: 22993618]
34. Slattery ML, John EM, Torres-Mejia G, Lundgreen A, Lewinger JP, et al. Angiogenesis genes, dietary oxidative balance, and breast cancer risk and progression: The breast cancer health disparities study. Int J Cancer. 2013
35. Slattery ML, Caan BJ, Duncan D, Berry TD, Coates A, et al. A computerized diet history questionnaire for epidemiologic studies. J Am Diet Assoc. 1994; 94:761–766. [PubMed: 8021418]
36. Murtaugh MA, Sweeney C, Giuliano AR, Herrick JS, Hines L, et al. Diet patterns and breast cancer risk in Hispanic and non-Hispanic white women: the Four-Corners Breast Cancer Study. Am J Clin Nutr. 2008; 87:978–984. [PubMed: 18400722]
37. Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, et al. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud publica de Mexico. 1998; 40:133–140. [PubMed: 9617194]
38. Horn-Ross PL, John EM, Lee M, Stewart SL, Koo J, et al. Phytoestrogen consumption and breast cancer risk in a multiethnic population: the Bay Area Breast Cancer Study. Am J Epidemiol. 2001; 154:434–441. [PubMed: 11532785]
39. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003; 164:1567–1587. [PubMed: 12930761]
40. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. [PubMed: 10835412]
41. Yu K, Li Q, Bergen AW, Pfeiffer RM, Rosenberg PS, et al. Pathway analysis by adaptive combination of P-values. Genetic epidemiology. 2009; 33:700–709. [PubMed: 19333968]
42. Kai Yu OL, William Wheeler. ARTP Gene and Pathway p-values computed using the Adaptive Rank Truncated Product. 2.0.0 ed. pp. R package. 2011
43. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity. 2005; 95:221–227. [PubMed: 16077740]
44. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American journal of human genetics. 2004; 74:765–769. [PubMed: 14997420]

45. Rodriguez F, Naderi S, Wang Y, Johnson CE, Foody JM. High prevalence of metabolic syndrome in young Hispanic women: findings from the national Sister to Sister campaign. Metab Syndr Relat Disord. 2013; 11:81–86. [PubMed: 23259587]
46. Sentell TL, He G, Gregg EW, Schillinger D. Racial/ethnic variation in prevalence estimates for United States prediabetes under alternative 2010 American Diabetes Association criteria: 1988–2008. Ethn Dis. 2012; 22:451–458. [PubMed: 23140076]
47. Romero CX, Romero TE, Shlay JC, Ogden LG, Dabelea D. Changing trends in the prevalence and disparities of obesity and other cardiovascular disease risk factors in three racial/ethnic groups of USA adults. Adv Prev Med. 2012; 2012:172423. [PubMed: 23243516]
48. Dorow DS, Devereux L, Dietzsch E, De Kretser T. Identification of a new family of human epithelial protein kinases containing two leucine/isoleucine-zipper domains. Eur J Biochem. 1993; 213:701–710. [PubMed: 8477742]
49. Slattery ML, John EM, Torres-Mejia G, Herrick JS, Giuliano AR, et al. Genetic variation in bone morphogenetic proteins and breast cancer risk in hispanic and non-hispanic white women: The breast cancer health disparities study. International journal of cancer Journal international du cancer. 2012
50. Kang Y. Pro-metastasis function of TGFbeta mediated by the Smad pathway. J Cell Biochem. 2006; 98:1380–1390. [PubMed: 16598746]
51. Lei X, Bandyopadhyay A, Le T, Sun L. Autocrine TGFbeta supports growth and survival of human breast cancer MDA-MB-231 cells. Oncogene. 2002; 21:7514–7523. [PubMed: 12386814]
52. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007; 447:1087–1093. [PubMed: 17529967]
53. Gaudet MM, Kuchenbaecker KB, Vijai J, Klein RJ, Kirchhoff T, et al. Identification of a BRCA2-specific modifier locus at 6p24 related to breast cancer risk. PLoS Genet. 2013; 9:e1003173. [PubMed: 23544012]
54. Milne RL, Gaudet MM, Spurdle AB, Fasching PA, Couch FJ, et al. Assessing interactions between the associations of common genetic susceptibility variants, reproductive history and body mass index with breast cancer risk in the breast cancer association consortium: a combined case-control study. Breast Cancer Res. 2010; 12:R110. [PubMed: 21194473]
55. Slattery ML, Lundgreen A, Wolff RK. MAP kinase genes and colon and rectal cancer. Carcinogenesis. 2012; 33:2398–2408. [PubMed: 23027623]
56. Slattery ML, Lundgreen A, Wolff RK. Dietary Influence on MAPK-Signaling Pathways and Risk of Colon and Rectal Cancer. Nutr Cancer. 2013; 65:729–738. [PubMed: 23859041]
57. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012; 486:400–404. [PubMed: 22722201]
58. Hori M, Inagawa S, Shimazaki J, Itabashi M, Hori M. Overexpression of mitogen-activated protein kinase superfamily proteins unrelated to Ras and AF-1 of estrogen receptor alpha mutation in advanced stage human breast cancer. Pathol Res Pract. 2000; 196:817–826. [PubMed: 11156322]

Nutr Cancer. Author manuscript; available in PMC 2016 January 28.

Table 1

Description of Study Population by self-reported Race/Ethnicity

	U.S. NHW					U. S. Hispanic/Native American or from Mexico				
	Controls		Cases		p value	Controls		Cases		p value
	N	%	N	%		N	%	N	%	
Total	1585	37.9	1481	41.2		2597	62.1	2111	58.8	
Study Site										
4-CBCS	1321	83.3	1227	82.8	NA[1]	723	27.8	597	28.3	NA
MBCS	0	0	0	0		994	38.3	816	38.7	
SFBCS	264	16.7	254	17.2		880	33.9	698	33.1	
Age (years)					NA					NA
<40	116	7.3	89	6.0		311	12	200	9.5	
40–49	408	25.7	409	27.6		831	32	713	33.8	
50–59	409	25.8	413	27.9		756	29.1	617	29.2	
60–69	349	22.0	361	24.4		526	20.3	430	20.4	
≥70	303	19.1	209	14.1		173	6.7	151	7.2	
Mean	56.6		56.0			52.3		52.7		
Menopausal Status					NA					NA
Pre-menopausal	494	31.5	489	33.5		1027	40.7	836	40.9	
Post-menopausal	1075	68.5	970	66.5		1499	59.3	1210	59.1	
Estimated Indigenous American Ancestry					NA					NA
Low (≤28%)	1577	99.5	1472	99.4		278	10.7	275	13.0	
Intermediate (>28 – 70%)	7	0.4	7	0.5		1686	64.9	1393	66.0	
High (>70%)	1	0.1	2	0.1		633	24.4	443	21.0	
ER/PR Status[2]										
ER+/PR+			695	68.2	NA			605	61.9	NA
ER+/PR−			121	11.9				115	11.8	
ER−/PR+			15	1.5				28	2.9	
ER−/PR−			188	18.4				229	23.4	
Alcohol Intake[3]										

	U.S. NHW					U. S. Hispanic/Native American or from Mexico				
	Controls		Cases		p value	Controls		Cases		p value
	N	%	N	%		N	%	N	%	
None	807	50.9	695	46.9	0.03	2025	78.0	1550	73.4	<.01
Any	778	49.1	786	53.1		572	22.0	561	26.6	
Cigarette Smoking										
Never	765	58.1	662	54.0	0.04	1627	71.9	1297	69.8	0.14
Ever	552	41.9	564	46.0		635	28.1	561	30.2	
Parity										
Nulliparous	248	15.7	261	17.6	<.01	181	7.0	229	10.8	<.01
1 to 2	638	40.3	646	43.6		790	30.5	786	37.2	
3 to 4	529	33.4	462	31.2		997	38.4	738	35.0	
≥5	167	10.6	111	7.5		626	24.1	358	17.0	
History of Diabetes or High Blood Sugar										
No	1299	91.4	1222	92.4	0.36	1945	83.4	1573	83.4	0.97
Yes	122	8.6	101	7.6		386	16.6	313	16.6	
	Median		Median			Median		Median		
BMI (kg/m^2)	25.8		25.7		0.76	29.4		28.5		<.01
Dietary Intake (per 1000 kcal)										
Calories (kcal)	1911.3		1947.4		0.12	2009.3		2168.1		<.01
Total Fat (g)	38.8		38.5		0.2	36.7		36.7		0.46
Fiber (g)[3]	10.7		10.8		0.99	12.9		12.5		<.01
Calcium (mg)	461.0		458.7		0.65	422.2		413.4		0.07
Folate (mcg)[3]	187.3		187.9		0.37	204.3		193.5		<.01
Vitamin C (mg)[3]	76.0		78.1		0.54	80.9		81.8		0.85
Vitamin E (mg)[3]	4.6		4.6		0.56	4.9		4.7		<.01
Beta Carotene[2,3] (mcg)	2290.1		2266.0		0.73	1997.5		1838.5		<.01

[1] p values not applicable (NA)

[2] Information unavailable for the MBCS.

[3] Included in the dietary oxidative balance score (DOBS)

Table 2

Associations between significant *MAPK* genes and breast cancer risk for all women and by level of Indigenous American Ancestry

	All					≤28%Indigenous Ancestry					> 28 – 70% Indigenous Ancestry					>70%Indigenous Ancestry					Interaction P-value
	Cn	Cs	OR[1]	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95%CI)	P_{ARTP}	(raw; adjusted)
JNK/ERK																				
MAP3K3 (rs3785574)					0.96					0.17					0.26					0.58	0.012,0.023
AA	1794	1555	1.00			880	798	1.00			692	583	1.00			222	174	1.00			
AG	1884	1607	1.00	(0.91, 1.10)		803	765	1.06	(0.92, 1.21)		773	647	1.00	(0.85, 1.16)		308	195	0.81	(0.62, 1.07)		
GG	472	407	1.03	(0.88, 1.19)		159	179	1.26	(1.00, 1.60)		214	160	0.88	(0.69, 1.11)		99	68	0.86	(0.59, 1.26)		
JNK																				
MAP3K9 (rs11628333)					0.02					0.51					0.06					0.04	0.018,0.087
TT/TC	3501	3080	1.00			1594	1505	1.00			1421	1210	1.00			486	365	1.00			
CC	648	488	0.86	(0.76, 0.98)		248	236	1.00	(0.83, 1.21)		257	180	0.83	(0.67, 1.02)		143	72	0.68	(0.49, 0.93)		
MAP3K9 (rs10483834)																					0.002,0.015
AA	2873	2372	1.00			1079	1038	1.00			1238	966	1.00			556	368	1.00			
AG/GG	1277	1197	1.09	(0.98, 1.20)		763	704	0.96	(0.84, 1.10)		441	424	1.23	(1.05, 1.45)		73	69	1.34	(0.93, 1.93)		
MAP3K9 (rs11622989)																					0.013,0.087
CC	1199	920	1.00			487	453	1.00			496	350	1.00			216	117	1.00			
CT/TT	2949	2649	1.16	(1.05, 1.29)		1353	1289	1.02	(0.88, 1.19)		1183	1040	1.25	(1.06, 1.47)		413	320	1.43	(1.09, 1.88)		
MAP3K9 (rs12883244)																					0.014,0.087
CC	1088	830	1.00			422	399	1.00			459	315	1.00			207	116	1.00			
CT/TT	3062	2739	1.16	(1.04, 1.29)		1420	1343	1.00	(0.85, 1.17)		1220	1075	1.27	(1.07, 1.50)		422	321	1.36	(1.03, 1.79)		
MAP3K9 (rs11158881)																					0.079,0.155
TT	2164	1814	1.00			1052	995	1.00			846	662	1.00			266	157	1.00			
TC/CC	1985	1752	1.08	(0.99, 1.19)		790	744	0.99	(0.87, 1.13)		832	728	1.12	(0.97, 1.30)		363	280	1.33	(1.03, 1.73)		
MAP3K9 (rs4902855)																					<.001,0.008
CC	1367	1092	1.00			578	554	1.00			555	407	1.00			234	131	1.00			
CT	2011	1817	1.13	(1.02, 1.25)		892	882	1.03	(0.89, 1.20)		821	716	1.17	(0.99, 1.38)		298	219	1.31	(0.99, 1.74)		
TT	772	660	1.07	(0.94, 1.22)		372	306	0.86	(0.71, 1.04)		303	267	1.21	(0.98, 1.50)		97	87	1.58	(1.09, 2.28)		
MAP3K9 (rs10143031)																					0.032,0.114

	All					≤28%Indigenous Ancestry					> 28 – 70% Indigenous Ancestry					>70%Indigenous Ancestry					Interaction P-value
	Cn	Cs	OR[1]	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95% CI)	P_{ARTP}	Cn	Cs	OR	(95%CI)	P_{ARTP}	(raw; adjusted)
CC	1114	1022	1.00			511	489	1.00			452	407	1.00			151	126	1.00			
CT	2087	1790	0.94	(0.84, 1.04)		914	866	1.00	(0.85, 1.17)		861	697	0.89	(0.75, 1.06)		312	227	0.87	(0.65, 1.17)		
TT	949	756	0.87	(0.76, 0.99)		417	387	0.98	(0.81, 1.18)		366	285	0.86	(0.69, 1.05)		166	84	0.62	(0.43, 0.89)		
MAP3K9 (rs11624934)																					0.078,0.155
AA/AG	3582	3174	1.00			1638	1565	1.00			1448	1236	1.00			496	373	1.00			
GG	568	394	0.80	(0.69, 0.91)		204	176	0.89	(0.72, 1.11)		231	154	0.79	(0.63, 0.98)		133	64	0.65	(0.47, 0.92)		
MAPK8 (rs10508901)					0.39					0.11					0.45					0.07	0.003,0.006
CC	2395	2043	1.00			824	830	1.00			1079	901	1.00			492	312	1.00			
CA/AA	1753	1525	0.96	(0.87, 1.05)		1018	911	0.90	(0.79, 1.03)		598	489	0.95	(0.81, 1.10)		137	125	1.41	(1.05, 1.87)		

[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, study center, BMI during referent year, parity, and genetic ancestry. Cn is controls and Cs is cases.

Nutr Cancer. Author manuscript; available in PMC 2016 January 28.

Table 3

Associations between significant *MAPK* genes and ER and PR tumor status[1]

	Cn	ER+ /PR+				ER+ / PR−				ER− / PR+				ER− / PR−				Multinomial p-value (raw; adjusted)
	N	N	OR[2]	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	
JNK/ERK																		
MAP3K3 (rs3785574)					0.96				0.93				0.76				0.002	0.011,0.019
AA	1418	597	1.00			106	1.00			21	1.00			156	1.00			
AG	1424	553	0.94	(0.82, 1.08)		113	1.09	(0.82, 1.43)		19	0.89	(0.48, 1.67)		196	1.25	(1.00, 1.57)		
GG	324	148	1.14	(0.91, 1.42)		16	0.71	(0.41, 1.22)		3	0.58	(0.17, 1.97)		63	1.74	(1.26, 2.39)		
JNK/p38																		
MAP3K7 (rs150117)					0.36				0.35				0.26				0.09	0.011,0.056
AA	1461	583	1.00			121	1.00			16	1.00			217	1.00			
AT	1380	572	1.04	(0.90, 1.19)		87	0.76	(0.57, 1.01)		18	1.21	(0.61, 2.39)		168	0.83	(0.67, 1.03)		
TT	323	143	1.09	(0.87, 1.35)		27	0.98	(0.63, 1.52)		9	2.71	(1.18, 6.22)		30	0.65	(0.43, 0.96)		
JNK																		
MAP3K9 (rs11622989)					0.01				0.45				0.79				0.36	0.027,0.209
CC	884	306	1.00			63	1.00			9	1.00			98	1.00			
CT/TT	2280	992	1.24	(1.07, 1.44)		172	1.04	(0.77, 1.41)		34	1.50	(0.72, 3.15)		317	1.27	(1.00, 1.61)		
MAP3K9 (rs17176971)																		0.082,0.463
GG	2054	901	1.00			155	1.00			30	1.00			281	1.00			
GA/AA	1112	396	0.82	(0.72, 0.95)		80	0.98	(0.74, 1.30)		13	0.79	(0.41, 1.52)		134	0.88	(0.71, 1.09)		
MAP3K9 (rs12883244)																		0.003,0.029
CC	789	260	1.00			65	1.00			8	1.00			86	1.00			
CT/TT	2377	1038	1.30	(1.11, 1.52)		170	0.84	(0.62, 1.13)		35	1.51	(0.70, 3.28)		329	1.29	(1.01, 1.66)		
MAP3K9 (rs4902855)																		0.018,0.172
CC	1030	372	1.00			88	1.00			11	1.00			123	1.00			
CT/TT	2136	926	1.18	(1.03, 1.36)		147	0.78	(0.59, 1.03)		32	1.45	(0.73, 2.90)		292	1.17	(0.93, 1.46)		
MAP3K9 (rs11624934)																		0.052,0.342
AA	1366	610	1.00			95	1.00			20	1.00			170	1.00			
AG	1413	564	0.91	(0.79, 1.04)		110	1.13	(0.85, 1.51)		18	0.87	(0.46, 1.65)		213	1.21	(0.98, 1.51)		
GG	387	123	0.73	(0.58, 0.91)		30	1.15	(0.75, 1.76)		5	0.83	(0.31, 2.25)		32	0.65	(0.44, 0.97)		

	Cn	ER+ /PR+				ER+ / PR−				ER− / PR+				ER− / PR−				Multinomial p-value (raw; adjusted)
	N	N	OR[2]	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	N	OR	(95% CI)	P_{ARTP}	
ERK																		
MAPK3 (rs7698)					0.85				0.048				0.77				0.88	0.373,0.373
CC	2660	1087	1.00			209	1.00			37	1.00			348	1.00			
CT/TT	502	210	1.01	(0.85, 1.21)		26	0.65	(0.43, 0.99)		6	0.87	(0.36, 2.07)		67	1.02	(0.77, 1.35)		

[1] Includes participants from 4-CBCS and SFBCS only.

[2] Odds Ratios (OR) and 95% Confidence Intervals adjusted for age, study center, BMI during referent year, parity and genetic ancestry. The pathway P_{ARTP} was of borderline significance for ER−/PR− tumors (P_{ARTP}=0.06)

Nutr Cancer. Author manuscript; available in PMC 2016 January 28.

Table 4

Interactions between *MAPK* genes and dietary intake

		Genotype (GT)[1]		GT1/High Diet		GT2/Low Diet		GT2/High Diet		Interaction P-value
	Pathway	1 (common)	2 (rare)	OR[2]	(95% CI)	OR	(95% CI)	OR	(95% CI)	raw, adjusted
Dietary Oxidative Balance Score (DOBS)										
DUSP4 rs2056025	DUSP	TT	TG/GG	0.85	(0.73, 1.00)	1.13	(0.92, 1.39)	0.73	(0.59, 0.90)	0.013, 0.053
MAP3K7 rs379912	JNK/p38	AA	AG/GG	0.74	(0.64, 0.85)	0.84	(0.66, 1.07)	0.93	(0.72, 1.21)	0.045, 0.223
MAP3K9 rs10483834	JNK	AA	GG	0.73	(0.62, 0.86)	0.72	(0.42, 1.23)	0.79	(0.44, 1.41)	0.032, 0.308
MAP3K9 rs17766621	JNK	TT	CC	0.72	(0.59, 0.86)	0.62	(0.45, 0.86)	0.94	(0.66, 1.34)	0.012, 0.122
MAPK14 rs7761118	p38	GG	GA/AA	0.76	(0.65, 0.87)	0.87	(0.68, 1.12)	0.87	(0.66, 1.14)	0.041, 0.290
MAPK1 rs2298432	ERK	CC	CA/AA	0.67	(0.55, 0.81)	0.89	(0.74, 1.07)	0.83	(0.68, 1.00)	0.018, 0.070
MAPK1 rs9610375	ERK	GG	TT	0.94	(0.75, 1.17)	1.07	(0.82, 1.39)	0.66	(0.50, 0.87)	0.048, 0.102
MAPK1 rs8136867	ERK	AA	GG	0.67	(0.53, 0.85)	0.81	(0.63, 1.06)	0.84	(0.63, 1.11)	0.034, 0.102
Calories										
DUSP4 rs12540995	DUSP	CC	TT	1.45	(1.18, 1.79)	0.65	(0.48, 0.87)	1.82	(1.39, 2.36)	0.012, 0.049
DUSP4 rs3824133	DUSP	AA	GG	1.47	(1.20, 1.81)	0.58	(0.42, 0.81)	1.83	(1.38, 2.42)	0.013, 0.049
DUSP4 rs567436	DUSP	AA	TT	1.56	(1.27, 1.91)	0.67	(0.50, 0.90)	1.99	(1.53, 2.59)	0.031, 0.061
MAP3K1 rs33323	JNK/ERK	CC	GG	1.89	(1.50, 2.40)	1.06	(0.81, 1.38)	1.40	(1.07, 1.82)	0.030, 0.149
MAPK8 rs10857565	JNK	GG	AA	1.76	(1.50,2.07)	0.86	(0.50, 1.49)	1.41	(0.87, 2.27)	0.029, 0.029
MAPK8 rs10508901	JNK	CC	AA	1.85	(1.56, 2.20)	1.10	(0.76, 1.57)	1.41	(0.97, 2.05)	0.007, 0.014
MAPK3 rs7698	ERK	CC	CT/TT	1.45	(1.26, 1.68)	0.74	(0.56, 0.97)	1.82	(1.41, 2.35)	0.005, 0.005
MAP3K2 rs12613413	JNK/ERK	TT	CC	0.91	(0.78, 1.06)	0.99	(0.60, 1.63)	1.71	(1.00, 2.94)	0.035, 0.091
Fat										
MAP3K2 rs6732279	JNK/ERK	TT	GG	0.75	(0.59, 0.95)	0.82	(0.63, 1.05)	0.86	(0.67, 1.10)	0.047, 0.091
Fiber										
MAPK8 rs10508901	JNK	CC	AA	0.73	(0.62, 0.87)	0.81	(0.58, 1.14)	0.88	(0.58, 1.33)	0.046, 0.093
MAPK1 rs2298432	ERK	CC	AA	0.71	(0.59, 0.86)	0.90	(0.66, 1.22)	1.02	(0.73, 1.42)	0.017, 0.034
MAPK1 rs743409	ERK	CC	TT	0.71	(0.57, 0.88)	0.88	(0.67, 1.14)	0.97	(0.73, 1.28)	0.027, 0.034
MAPK1 rs9610375	ERK	GG	TT	1.04	(0.83, 1.30)	1.08	(0.84, 1.40)	0.67	(0.51, 0.90)	0.005, 0.019
MAPK1 rs8136867	ERK	AA	GG	0.70	(0.55, 0.87)	0.81	(0.62, 1.04)	0.94	(0.72, 1.23)	0.009, 0.027
Calcium										

		Genotype (GT)[1]		GT1/High Diet		GT2/Low Diet		GT2/High Diet		Interaction P-value
	Pathway	1 (common)	2 (rare)	OR[2]	(95% CI)	OR	(95% CI)	OR	(95% CI)	raw, adjusted
						Dietary Oxidative Balance Score (DOBS)				
MAPK1 rs8136867	ERK	AA	GG	0.75	(0.60, 0.95)	0.83	(0.64, 1.08)	0.98	(0.75, 1.28)	0.018, 0.071
						Folate				
DUSP4 rs2056025	DUSP	TT	GG	0.85	(0.73, 0.99)	1.27	(0.66, 2.43)	1.11	(0.58, 2.10)	0.035, 0.139
MAP3K7 rs379912	JNK/p38	AA	GG	0.72	(0.63, 0.84)	0.74	(0.31, 1.79)	0.88	(0.38, 2.03)	0.038, 0.191
MAP3K9 rs8011507	JNK	AA	GG	0.71	(0.61, 0.83)	0.33	(0.17, 0.67)	1.06	(0.45, 2.47)	0.009, 0.100
MAP3K9 rs11622989	JNK	CC	TT	0.98	(0.76, 1.26)	1.43	(1.11, 1.85)	0.92	(0.71, 1.20)	0.021, 0.183
MAP3K9 rs12883244	JNK	CC	TT	1.00	(0.77, 1.30)	1.37	(1.06, 1.77)	0.93	(0.72, 1.22)	0.043, 0.285
MAP3K9 rs17766621	JNK	TT	CC	0.69	(0.57, 0.83)	0.69	(0.51, 0.93)	0.68	(0.48, 0.96)	0.050, 0.285
MAPK8 rs10508901	JNK	CC	AA	0.66	(0.55, 0.79)	0.78	(0.56, 1.08)	0.91	(0.63, 1.33)	0.006, 0.012
MAPK14 rs7761118	p38	GG	AA	0.72	(0.63, 0.83)	0.88	(0.28, 2.75)	2.14	(0.41, 11.13)	0.016, 0.113
MAPK14 rs3730327	p38	AA	GG	0.73	(0.63, 0.84)	0.90	(0.31, 2.60)	2.15	(0.41, 11.17)	0.030, 0.182
MAPK1 rs2298432	ERK	CC	AA	0.64	(0.53, 0.77)	0.87	(0.63, 1.19)	0.85	(0.61, 1.20)	0.009, 0.036
MAPK1 rs743409	ERK	CC	TT	0.68	(0.55, 0.85)	0.88	(0.67, 1.15)	0.91	(0.68, 1.21)	0.043, 0.087
MAPK1 rs8136867	ERK	AA	GG	0.68	(0.54, 0.86)	0.80	(0.62, 1.04)	0.92	(0.69, 1.21)	0.016, 0.049

[1] Referent group is genotype group 1 (GT1) or homozygote common genotype and low dietary intake; Genotype 2 group designated as GT2 represents homozygote rare genotype or in some instances the dominant model as indicated.

[2] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, genetic ancestry, study center, BMI (kg/m^2) during referent year, and parity.

Nutr Cancer. Author manuscript; available in PMC 2016 January 28.

Table 5

Interactions between *MAPK* genes and BMI, alcohol intake, cigarette smoking, and self-reported history of diabetes

		Genotype (GT)[1]		GT1/High Lifestyle		GT2/Low lifestyle		GT2/High Lifestyle		Interaction P-value
	Pathway	1 (common)	2 (rare)	OR[2]	(95% CI)	OR	(95% CI)	OR	(95% CI)	raw, adjusted
Pre-Menopausal Women BMI (<25 kg/m^2 vs. ≥30 kg/m^2)										
DUSP4 rs474824	DUSP	CC	TT	0.48	(0.33,0.69)	0.70	(0.48,1.01)	0.62	(0.41,0.93)	0.032,0.128
MAP3K9 rs11625206	JNK	CC	TT	0.93	(0.70,1.23)	1.59	(1.03,2.43)	0.76	(0.48,1.19)	0.019,0.181
MAP3K9 rs10143031	JNK	CC	TT	0.96	(0.67,1.37)	1.44	(1.00,2.07)	0.79	(0.54,1.15)	0.033,0.248
MAP3K9 rs17766621	JNK	TT	CC	0.68	(0.52,0.89)	0.55	(0.35,0.86)	1.04	(0.59,1.83)	0.046,0.299
MAP3K9 rs8022269	JNK	GG	AA	0.99	(0.71,1.38)	1.50	(1.04,2.17)	0.76	(0.51,1.14)	0.014,0.153
MAP3K9 rs11624934	JNK	AA	GG	0.87	(0.65,1.16)	1.39	(0.92,2.12)	0.62	(0.40,0.95)	0.045,0.299
MAPK8 rs10857565	JNK	GG	AA	0.67	(0.53,0.84)	0.36	(0.17,0.75)	0.66	(0.24,1.81)	0.033,0.065
MAPK1 rs2298432	ERK	CC	AA	0.58	(0.44,0.76)	0.69	(0.44,1.07)	0.70	(0.43,1.15)	0.012,0.049
MAPK1 rs743409	ERK	CC	TT	0.60	(0.44,0.81)	0.72	(0.49,1.05)	0.85	(0.56,1.28)	0.022,0.049
Post-Menopausal Women BMI (<25 kg/m^2 vs. ≥30 kg/m^2)										
MAP3K1 rs33330	JNK/ERK	GG	AA	0.81	(0.66,0.99)	0.74	(0.51,1.08)	1.23	(0.83,1.81)	0.035,0.173
MAP3K3 rs3785574	JNK/ERK	AA	GG	1.06	(0.85,1.32)	1.40	(0.97,2.03)	0.76	(0.54,1.06)	0.006,0.010
MAP3K9 rs1034769	JNK	TT	TG/GG	0.98	(0.83,1.16)	1.23	(0.94,1.61)	0.78	(0.61,0.99)	0.009,0.098
MAPK14 rs3804454	p38	AA	CC	0.79	(0.66,0.94)	1.01	(0.59,1.72)	1.19	(0.66,2.16)	0.018,0.125
MAPK14 rs17714205	p38	CC	CT/TT	0.83	(0.70,0.98)	0.92	(0.70,1.22)	1.13	(0.87,1.46)	0.030,0.183
Alcohol Intake (none vs. >75% of drinkers)[3]										
DUSP4 rs474824	DUSP	CC	TT	1.65	(1.19, 2.29)	1.07	(0.90, 1.27)	1.05	(0.78, 1.41)	0.014, 0.055
MAP3K1 rs33330	JNK/ERK	GG	AA	1.36	(1.09, 1.71)	1.31	(1.02, 1.69)	1.19	(0.75, 1.88)	0.044, 0.218
MAP3K7 rs150117	JNK/ERK	AA	TT	1.30	(1.03, 1.65)	1.22	(1.00, 1.49)	1.36	(0.88, 2.11)	0.049, 0.243
MAP3K9 rs11622989	JNK	CC	TT	1.35	(0.99, 1.85)	1.28	(1.09, 1.50)	1.25	(0.90, 1.75)	0.033, 0.320
MAP3K9 rs12883244	JNK	CC	TT	1.43	(1.03, 1.98)	1.25	(1.07, 1.47)	1.24	(0.90, 1.72)	0.022, 0.236
Cigarette Smoking (Never vs. Ever)[4]										
MAP3K9 rs11622989	JNK	CC	TT	1.21	(0.99, 1.47)	1.29	(1.09, 1.54)	1.05	(0.84, 1.30)	0.011, 0.111
MAP3K9 rs12883244	JNK	CC	TT	1.22	(0.99, 1.50)	1.23	(1.03, 1.46)	1.07	(0.87, 1.33)	0.028, 0.237
MAP3K9 rs11624934	JNK	AA	GG	0.95	(0.81, 1.11)	0.74	(0.61, 0.89)	0.95	(0.73, 1.24)	0.027, 0.237
MAPK12 rs2272857	p38	GG	AA	1.23	(1.07, 1.42)	1.26	(0.97, 1.64)	1.33	(0.93, 1.91)	0.043, 0.085

		Genotype (GT)[1]		GT1/High Lifestyle		GT2/Low lifestyle		GT2/High Lifestyle		Interaction P-value
	Pathway	1 (common)	2 (rare)	OR[2]	(95% CI)	OR	(95% CI)	OR	(95% CI)	raw, adjusted
MAPK14 rs13196204	p38	TT	GG	1.02	(0.91, 1.15)	0.88	(0.57, 1.35)	1.82	(1.04, 3.19)	0.036, 0.255
						History of Diabetes (No vs. Yes)[5]				
MAP3K9 rs11625206	JNK	CC	TT	1.40	(1.12,1.74)	1.01	(0.85,1.20)	0.71	(0.47,1.07)	0.001,0.011
MAP3K9 rs11844774	JNK	TT	CC	0.95	(0.73,1.23)	1.02	(0.88,1.18)	1.57	(1.16,2.13)	0.026,0.057
MAP3K9 rs11628333	JNK	TT	CC	1.46	(1.15,1.85)	0.94	(0.80,1.11)	0.90	(0.63,1.29)	0.009,0.057
MAP3K9 rs11622989	JNK	CC	TT	0.92	(0.70,1.21)	1.05	(0.91,1.22)	1.62	(1.19,2.21)	0.017,0.057
MAP3K9 rs12883244	JNK	CC	TT	0.89	(0.67,1.17)	1.00	(0.86,1.16)	1.67	(1.24,2.26)	0.003,0.020
MAP3K9 rs1115881	JNK	TT	CC	0.94	(0.77,1.15)	0.97	(0.80,1.18)	1.85	(1.18,2.90)	0.010,0.057
MAP3K9 rs4902855	JNK	CC	TT	0.93	(0.72,1.19)	0.96	(0.82,1.11)	1.62	(1.17,2.24)	0.009,0.057
MAP3K9 rs10143031	JNK	CC	TT	1.37	(1.05,1.79)	0.93	(0.80,1.08)	0.81	(0.60,1.10)	0.028,0.057
MAP3K9 rs17108548	JNK	TT	CC	0.95	(0.79,1.15)	0.91	(0.71,1.15)	1.73	(1.03,2.91)	0.015,0.057
MAP3K9 rs8022269	JNK	GG	AA	1.52	(1.17,1.96)	1.04	(0.89,1.20)	0.85	(0.61,1.18)	0.002,0.017
MAP3K9 rs11624934	JNK	AA	GG	1.46	(1.16,1.84)	0.88	(0.74,1.04)	0.75	(0.51,1.10)	0.002,0.020
MAPK1 rs9610470	ERK	TT	CC	1.20	(1.01,1.42)	1.14	(0.86,1.50)	0.69	(0.32,1.45)	0.019,0.074

[1] Referent group is the common genotype group 1 (GT1) and low risk exposure (i.e. BMI <25kg/m^2, no alcohol intake, never smoker, no history of diabetes); GT2 is genotype 2 group that represents homozygote rare genotype or in some instances the dominant model as indicated.

[2] Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, genetic ancestry, study center, BMI during referent year, and parity.

[3] To determine top quarter of drinkers we used the following study specific cut-points: 4-CBCS 10.45 g/day, MBCS 4.11 g/day, and SFBCS 10.86 g/day.

[4] Smoking information is missing from 5 women from the 4-CBCS and was not collected for 1095 women from the SFBCS.

[5] Diabetes information is missing from 72 women from the 4-CBCS, 152 women from the MBCS, and was not collected for 584 women from the SFBCS.